



NATURAL FOOD TRACER : A NEW SYSTEM FOR ANIMAL PRODUCTS TRACEABILITY AND AUTHENTICATION

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One of the most relevant characteristics of the Italian food production system is the presence of a wide range of typical products.



In the Italian national list of DM 350/1999 there are at present 1,424 typical animal products: 689 derived from meat, 585 derived from milk and 98 derived from fish.

The general picture of the data concerning quantity and type of certified products (POD and PGI) for each European country underlines the huge, if not delicate, importance of this branch of economic activity.

As we can see in the next table every European country has some typical certified European products POD or PGI.

TYPICAL EUROPEAN CERTIFIED PRODUCTS



In particular Italy and France are the leading producers of the largest quantities of these products.

UE	CHEESE	MEAT	OIL	FRUIT	FRESH	BAKERY	OLIVE	OTHERS	
COUNTRY	PRODUCTS	& FAT	& CEREAL	MEAT	PRODUCTS				TOTAL
Belgium	1	2	1			1		5	10
Cech Rep.						3		6	9
Denmark	2			1					3
Germany	4	8	1	3	3	4		46	69
Greece	20		26	22		1	10	6	85
Spain	19	11	20	34	13	8		9	114
FRANCE	45	4	9	26	51	2	3	15	155
Ireland	1	1			1			1	4
ITALY	34	29	38	51	2	3	2	7	166
Luxembourg		1	1		1			1	4
Low countries	4			2					6
Austria	6	2	1	3					12
Portugal	12	28	8	20	27		1	10	105
Finland				1		2		1	4
Sweden	2	1				1			4
UK	12			1	8			10	30

This sector we consider to be threatened by common malpractices.

Italy and France are the nations in which the phenomena of imitation and falsification are the most widespread.

Italian statistics report that food piracy leads to an annual loss of **2,500 billion euros for Italian agriculture**

Especially in recent years, there are numerous and repeated attempts at imitation and falsification of typical national products in foreign markets.

For example:



Parmesao (Brasil),
Regianito (Argentina),
Danish Grana (USA)



Parma Ham (USA),
Daniele Ham & C.
(USA)



Mozzarella
company by
Dallas (USA)

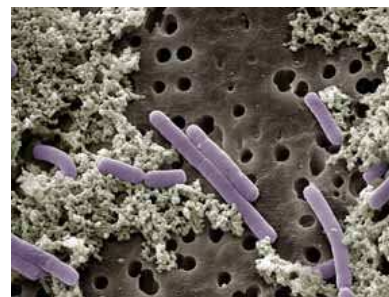
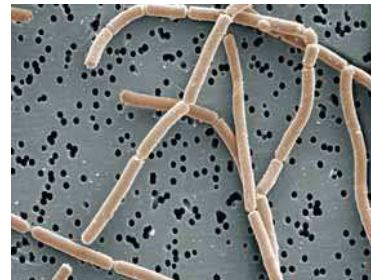


Asiago by
Wisconsin
(USA)

The development of economic and efficient traceability / authentication systems is extremely important in order to safeguard the authenticity of these typical products.

To face this pressing contemporary problem we have developed a system based on the combined use of **NATURAL TRACER** and **DNA** analysis.

NATURAL TRACER: every natural product (vegetable, animal or microbic) that we can use to unequivocally trace a food product.



This new traceability system can:

- **genetically characterize an ingredient already present in the typical product (such as pepper in salami)**
- **use very small amounts of a tracer (vegetable, animal or microbic), appropriately selected and processed, in order to “imprint”, i.e. label, the agro-food product.**

The actual identity of the tracer used as well as its DNA profile, naturally remains confidential, in order to prevent imitation.

The application of small amount of natural tracer on a food product will not lead to any modification of organoleptic and commercial characteristics of the product itself.

This approach will meet with the consensus of both the consumer and the producer alike.

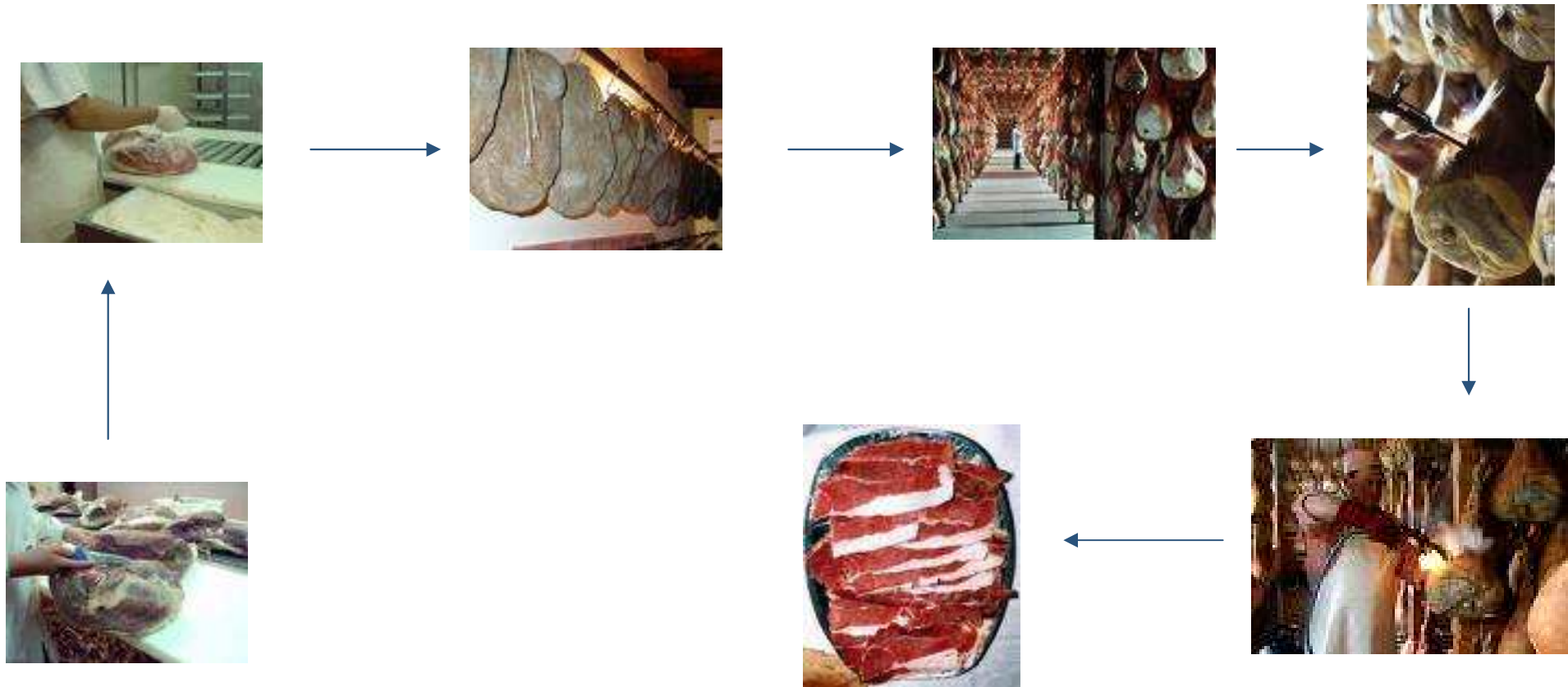
The authenticity of a commercial product, sold with a particular trademark, can therefore be verified by means of DNA analysis.

The DNA fingerprinting of the tracer used to mark the product must coincide unmistakably with the DNA fingerprinting of the tracer itself present in a database.

A comparison reveals the perfect overlap between the two.

Italian, European, American, China Patent pending

A trial was conducted on the new system in the dry - cured ham production chain.



- **STEP 1: CHOICE OF TRACER SPECIES**
- **STEP 2: CHOICE OF VARIETY**
- **STEP 3: CHOICE OF MICROSATELLITES (SSR)**
- **STEP 4: OPTIMIZATION OF SSR ANALISYS**
- **STEP 5: GENETIC CHARACTERIZATION OF VARIETY**
- **STEP 6: CHOICE OF TRACER SYSTEM**
- **STEP 7: APPLICATION OF TRACER TO HAM**

These factors should be consider:

- **easy preservation**
- **autogamous or agamous species**
- **genetic variability**
- **presence of scientific publications**

Some vegetable species such as rice, wheat and olive were considered. Then **WHEAT** was selected because:



- It's an autogamous species: it contains a low amount of “outsider” seeds in the seeds lot
- Many studies have shown that this species have a high genetic variability easily evaluated by means of DNA analysis.
- It's already a component of dry – cured ham fat preparation

We selected:

- **PURE UNIQUE LINE**
- **OLD VARIETIES**
- **NOT COMMERCIAL SELECTIONS**



23 wheat varieties were chosen:

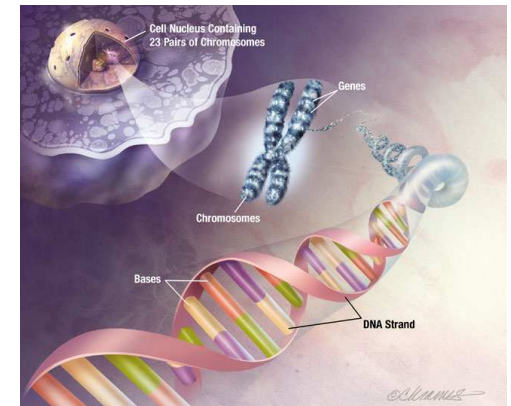
11 common wheats (GT 1 → GT 11)

12 durum wheats (GD 1 → GD 12)

STEP 3: CHOISE OF MICROSATELLITES (SSR)

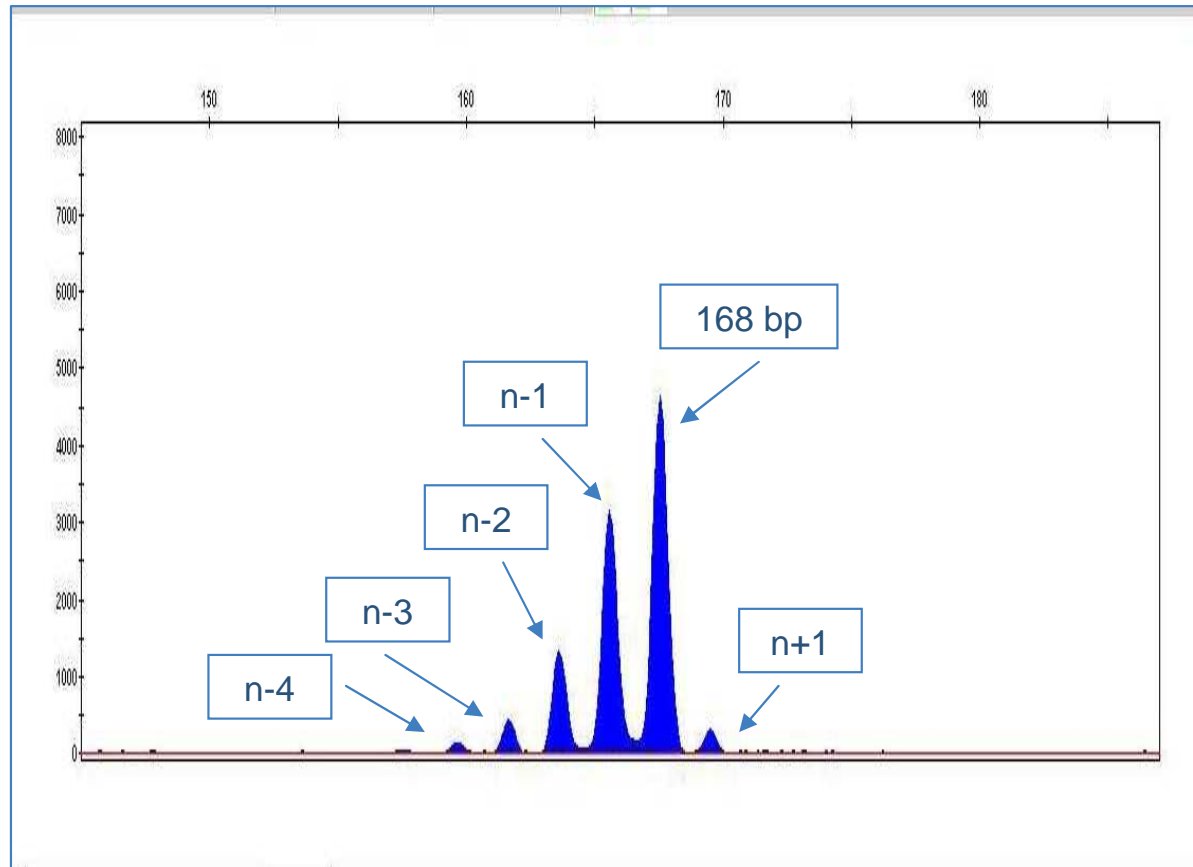
Consideration was given to the following factors:

- Allele number > 7
- SSR were located on different chromosomes
- Diversity index value > 0.5
- Allele size: 100 - 300 bp
- Annealing temperature



9 SSR were chosen: SSR 1 → SSR9

STEP 4: OPTIMIZATION SSR ANALISYS



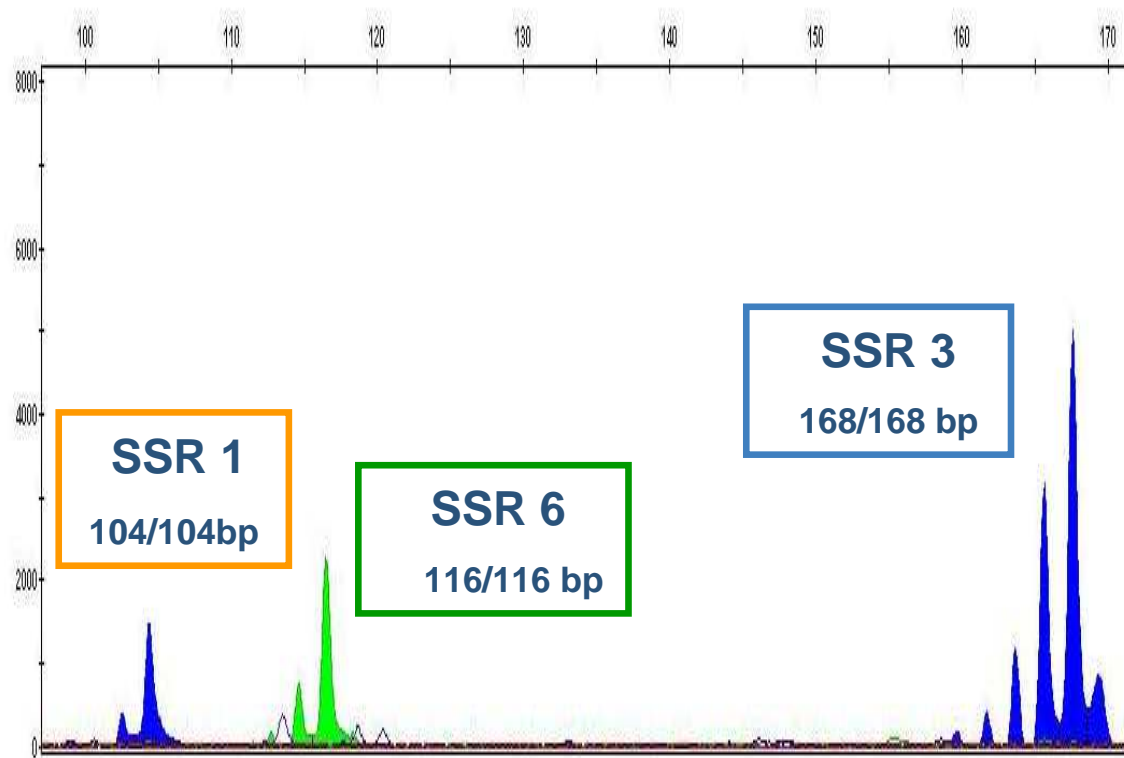
**Only 7 SSR
produced a good
electropherogram
with a strong
fluorescent signal**

variety: GT1

microsatellites: SSR 3

genotype: 168/168

To save money and time multiplex PCR were developed



GT2 variety was characterized at the same time with:

- **SSR 1**
- **SSR 3**
- **SSR 6**

STEP 5: GENETIC CHARACTERIZATION OF WHEAT VARIETIES

A database was created with fingerprinting profiles of these **19 possible FOOD TRACERS**

varietà	SSR 1	SSR 2	SSR 3	SSR 4	SSR 5	SSR 6	SSR 7
GT 1	a	a	a	a	a	a	a
GT 2	b	b	a	b	b	a	b
GT 3	b	c	b	c	b	b	c
GT 4	c	a	b	d	b	c	d
GT 5	b	b	a	a	b	c	e
GT 6	a	b	b	e	b	a	c
GT 7	a	d	b	f	b	a	b
GT 8	c	a	a	e	b	c	a
GT 9	a	e	c	e	b	a	e
GT 10	a	a	a	d	b	c	e
GT 11	a	a	b	d	c	d	d
GD 1	d	f	d	g	d	b	f
GD 2	e	f	d	f	h	b	g
GD 3	b	g	d	h	b	a	h
GD 4	f	h	e	h	e	b	d
GD 5	b	g	d	f	b	a	g
GD 6	b	g	d	f	b	a	g
GD 7	g	g	d	i	b	a	i
GD 8	e	f	d	f	f	b	i
GD 9	e	g	d	b	g	a	g
GD 10	e	f	a	f	f	b	l
GD 11	e	f	a	f	f	b	m
GD 12	e	f	a	f	f	b	m

Two tracer systems were considered:



- fat to grease the pork leg



- ink to mark the pork leg

STEP 6: CHOICE OF TRACER SYSTEM



FAT

WHEAT
(GT2)

MIX



TEST *in vitro*

- Different concentrations 10%, 1%, 0,1%
- Methods to isolate DNA from this matrix were developed
- Amplificability of DNA in different maturation time points was detected

STEP 6: CHOISE OF TRACER SYSTEM



INK

WHEAT
(GT2)

MIX



TEST *in vitro*

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STEP 7: APPLICATION OF TRACER TO HAM



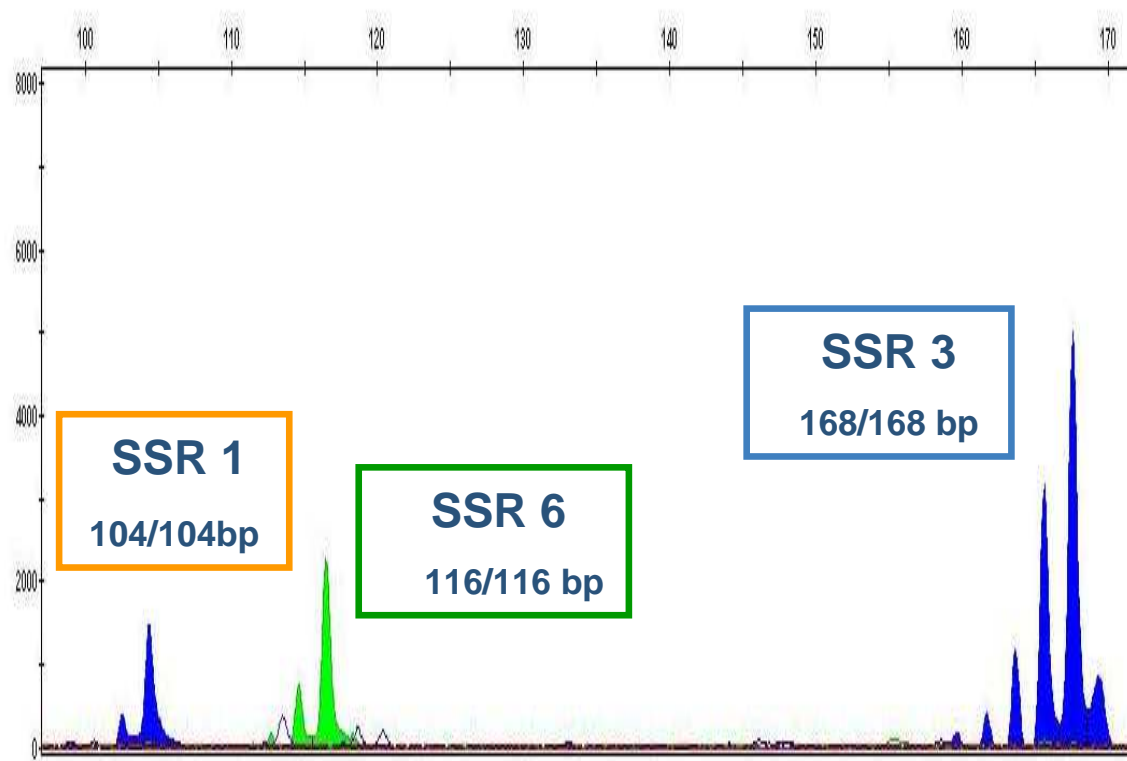
The ink commonly used to mark the green legs were mixed with wheat flour of line GT2.

STEP 7: APPLICATION OF TRACER TO HAM



**The ink were used to mark 5 green legs.
At different time points from the application of the tracers, a scratch of the labelled / marked region of the legs was recovered, DNA was isolated and amplified with the same microsatellites.**

The corresponding wheat DNA was identified at the different maturation time points and up to 14 months after application on ham.



The genotype profile of tracer used to mark the ham consistently matched its corresponding fingerprinting present in the database.

- **The system is simple, fast and economic**
- **Possibilities of imitation are therefore non-existent since vegetable varieties, each with its specific fingerprinting, are innumerable**
- **Application of natural food tracer will not lead to any modification of organoleptic and commercial characteristics of the product itself. In other words the product remains perfectly intact.**
- **Modifications of this system can also be applied to all available commercial products.**